THE THALLUS AND SPORE DEVELOPMENT OF *LOBOSPIRA BICUSPIDATA* ARESCHOUG (DICTYOTALES: PHAEOPHYTA)

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Summary

EDELSTEIN, TIKVAH, & WOMERSLEY, H. B. S. (1975).—The thallus and spore development of *Lobospira bicuspidata* Areschoug (Dictyotales: Phaeophyta). *Trans. R. Soc. S. Aust.* **99**(3), 149-156, 30 August, 1975.

The apical growth of *Lobospira bicuspidata*, release of tetraspores, and growth of the spores in culture to plants up to 1 cm across, are described. Both development of the axes and growth of the sporelings is from a marginal row of apical cells, and the thallus is monopodially developed. *Lobospira* is therefore placed in the Zonarieae group of the Dictyotales.

Introduction

Lobospira bicuspidata Areschoug is a distinctive brown alga referred to the Dictyotales. It occurs from Nickol Bay, Western Australia, around southern Australia to Eden, N.S.W., and around Tasmania (Womersley 1967, p. 215) and is frequently abundant in regions of moderate to strong water movement, from just below low tide level to 35 m deep.

The alga (Fig. 2*A*) is easily recognized by its spirally twisted axes, with a phyllotaxis of about 1/3, bearing laterals with bicuspid, determinate ramuli (Harvey 1858, pl. 34), and with lower branches bearing recurved attachment tendrils. Kjellman (1897, pp. 295, 297) and Oltmanns (1922, p. 185) considered that the thallus develops from an apical cell, with sympodial branching, and *Lobospira* has thus been considered as a member of the Dictyoteae. The sporangia, about 100 μm in diameter, occur scattered over the thallus (Fig. 2*B*); they are developed from cortical cells and sunken in the thallus (Fig. 1*B*). Neither division of the sporangia nor release of spores has been previously reported, and release was only obtained by the present authors on the one occasion. Sexual reproductive cells also have never been observed. While *Lobospira* has usually been placed in the Dictyotales, and the Dictyoteae (Womersley 1967, p. 215), its relationships have not been established.

This paper reports observations on apical development, spore release and early growth of the thallus, made in 1972 while the first author was on leave at the University of Adelaide.

Methods

Plants (ADU, A42264) were collected in drift at Aldinga reef, South Australia, on 27 May, 1972, and transferred to the laboratory in sea water. The specimens (Fig. 2*B*) bore mature sporangia, many of which released tetrads of spores. Fertile branches were placed in a glass jar with Provasoli ES medium (Starr 1971, p. 359) in a 15°C culture room, and spores allowed to settle on slides during the next two days. On day 3 the slides with attached sporelings were transferred to petri dishes (5 cm in diameter), and germanium dioxide at a concentration of 5 p.p.m. added to the medium. Single sporelings from the slides were detached to be grown in free culture; they were washed several times in a well-slide and inoculated into a new set of dishes each with 15-18 sporelings. After 4 weeks, cultures were maintained in SWM 3 medium (Chen, Edelstein & McLachlan 1969).

As the sporelings developed, considerable difficulty occurred with bacterial (and at one stage fungal) contamination. Addition of penicillin to the Provasoli medium had little effect, but streptomycin (100-150 mg streptomycin sulphate/l of seawater) eliminated most of the bacteria, and a commercial fungicide, Mycostatin-Dusting Powder (1,000,000 units) (E. R. Squibb & Sons Ltd, Melbourne) proved

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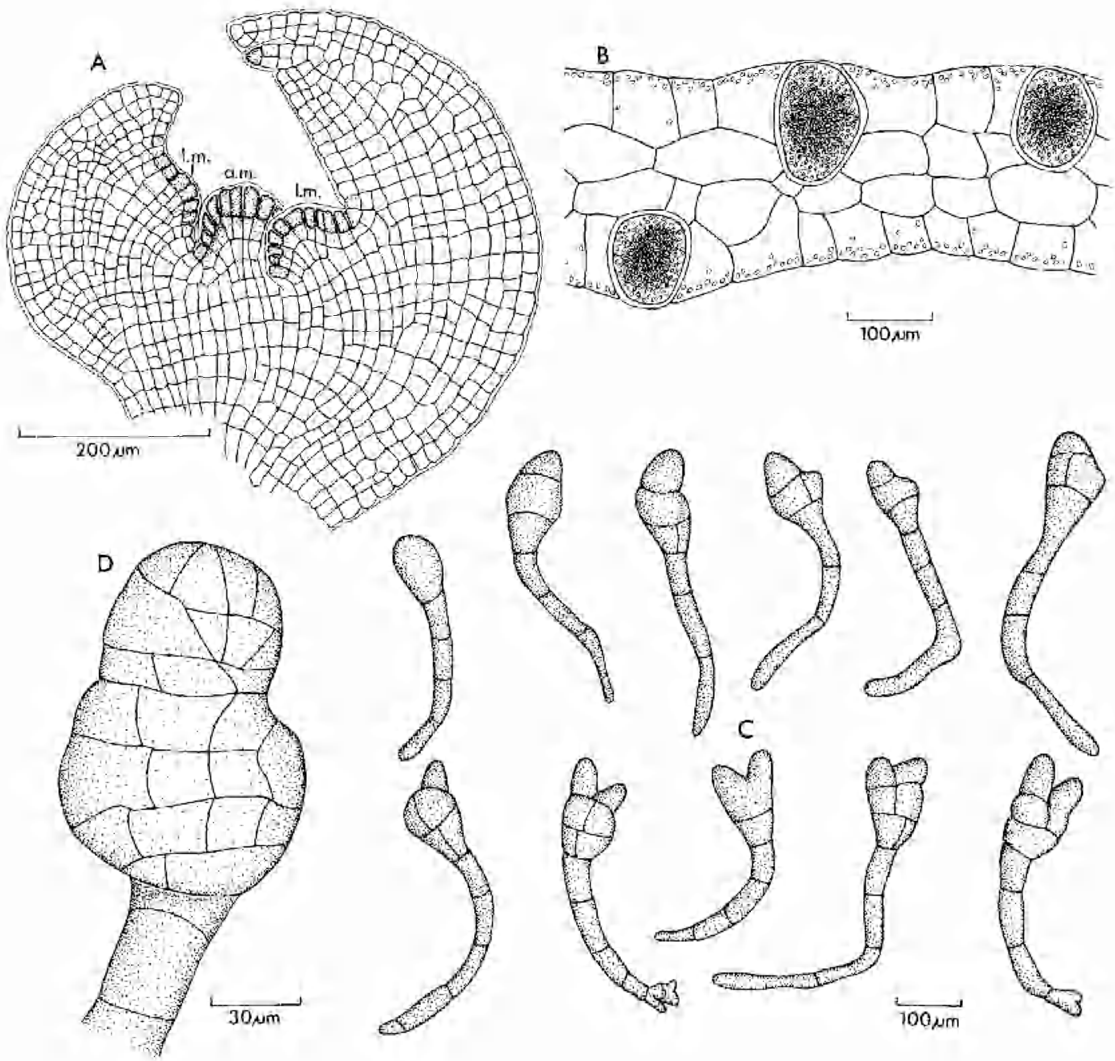

Fig. 1. *A*. Apical development, with two young laterals present, the older one (right) becoming bicuspid. The axis meristem (a.m.) continues growth of the branch, and the lateral meristems (l.m.) may or may not develop further into laterals (ADU, A42264).
B. Cross section of ramulus bearing sporangia (ADU, A42264).
C. Various sporelings 1 week old, with a rhizoid 3–5 cells long and early stages of the "cell-mass".
D. A sporeling 3 weeks old, with a well developed cell-mass.

to be effective when used as a single treatment of 200 mg of "Mycostatin" per 100 ml of sea water for 20 hours. Repeated treatments with streptomycin and Mycostatin were used and frequent cleaning of the sporelings with glass needles was carried out. While this damaged some sporelings, the majority survived and continued to develop to plants consisting of clusters of branches up to 1 cm across, but from which it was impossible to clean the epiphytes.

In general, cultures were maintained at 15°C under a light intensity of 600-800 lux provided by 40 watt white fluorescent lights, and a regime of 14 hrs light/10 hrs dark. The me-

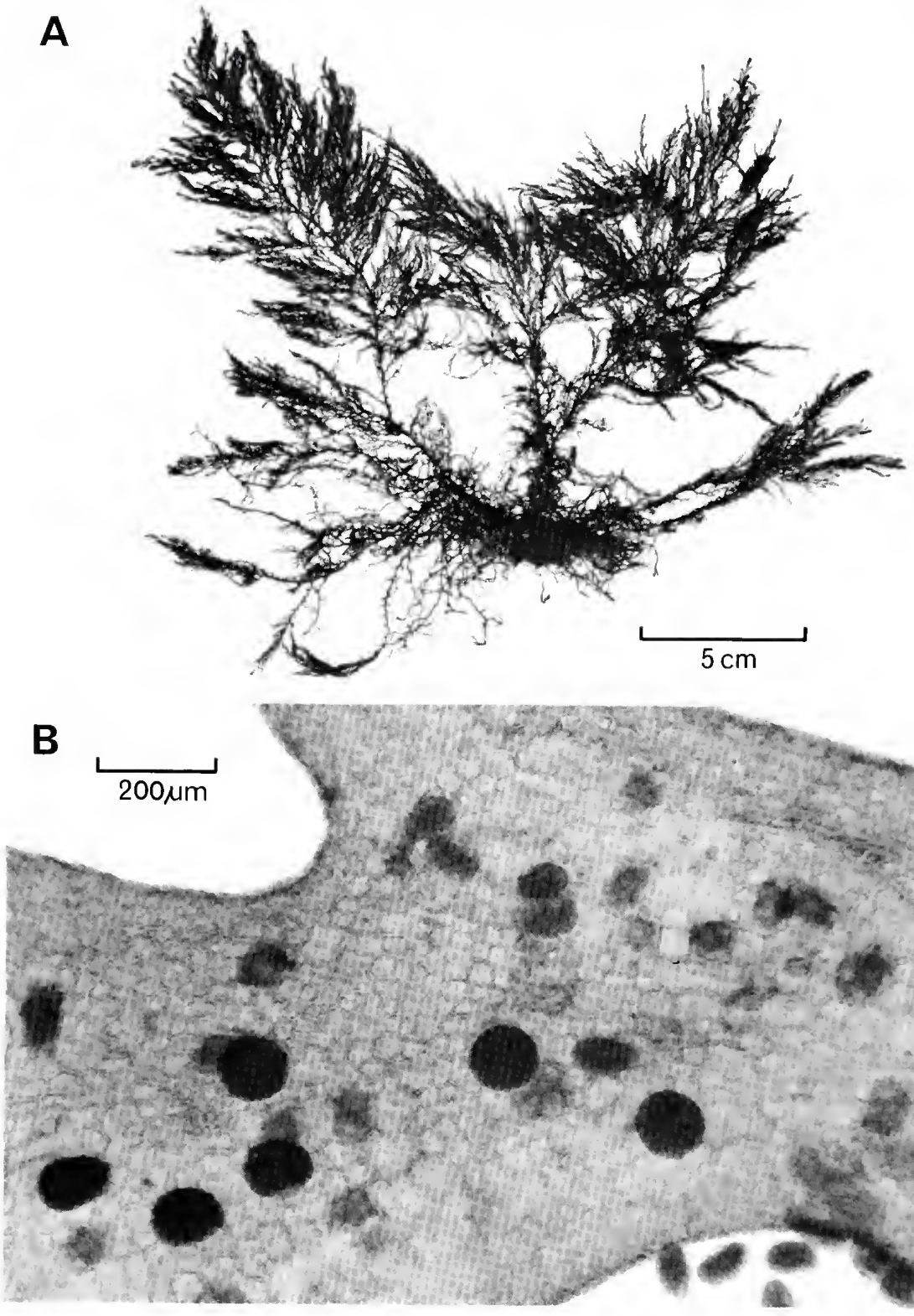

Fig. 2.

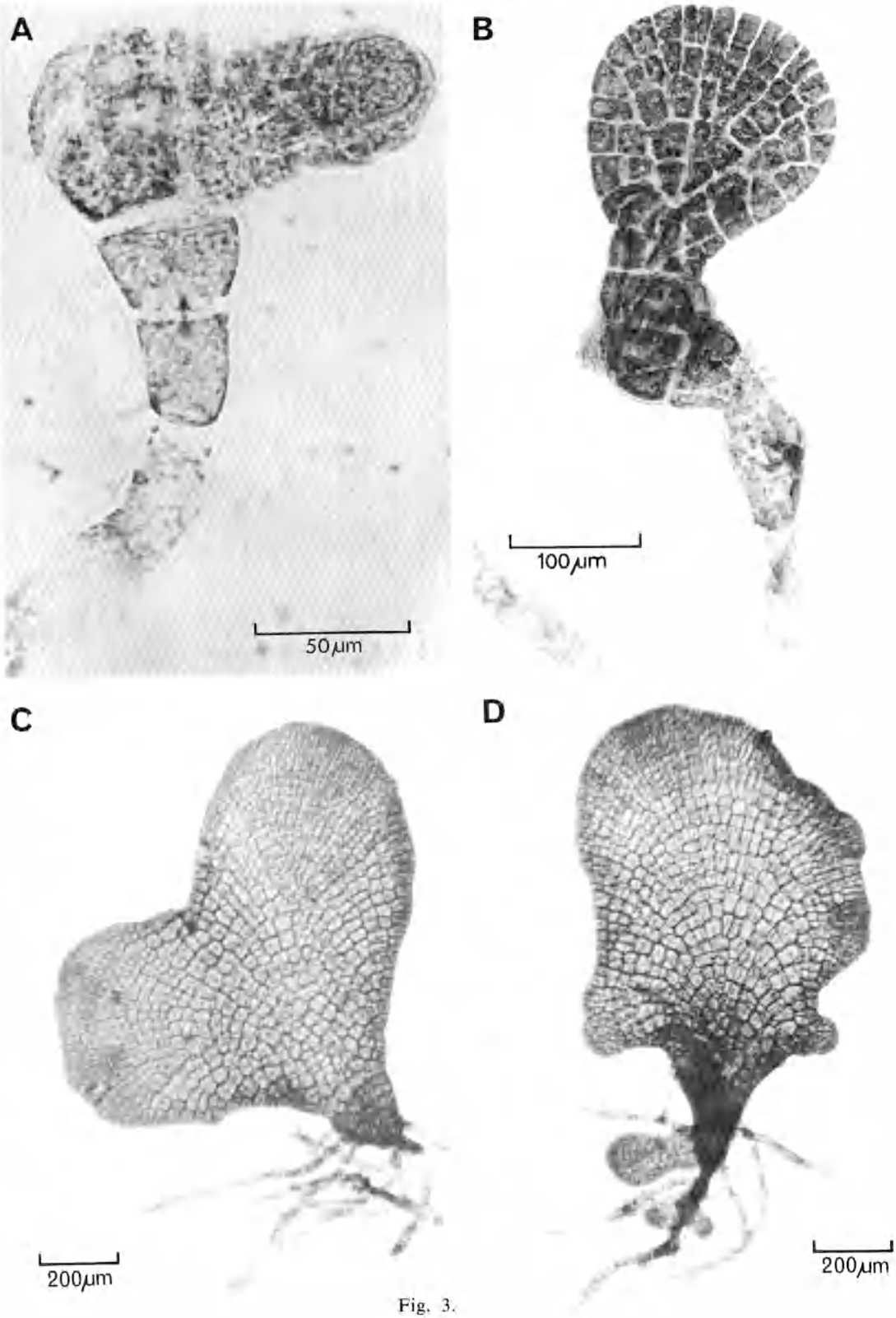

Fig. 3.

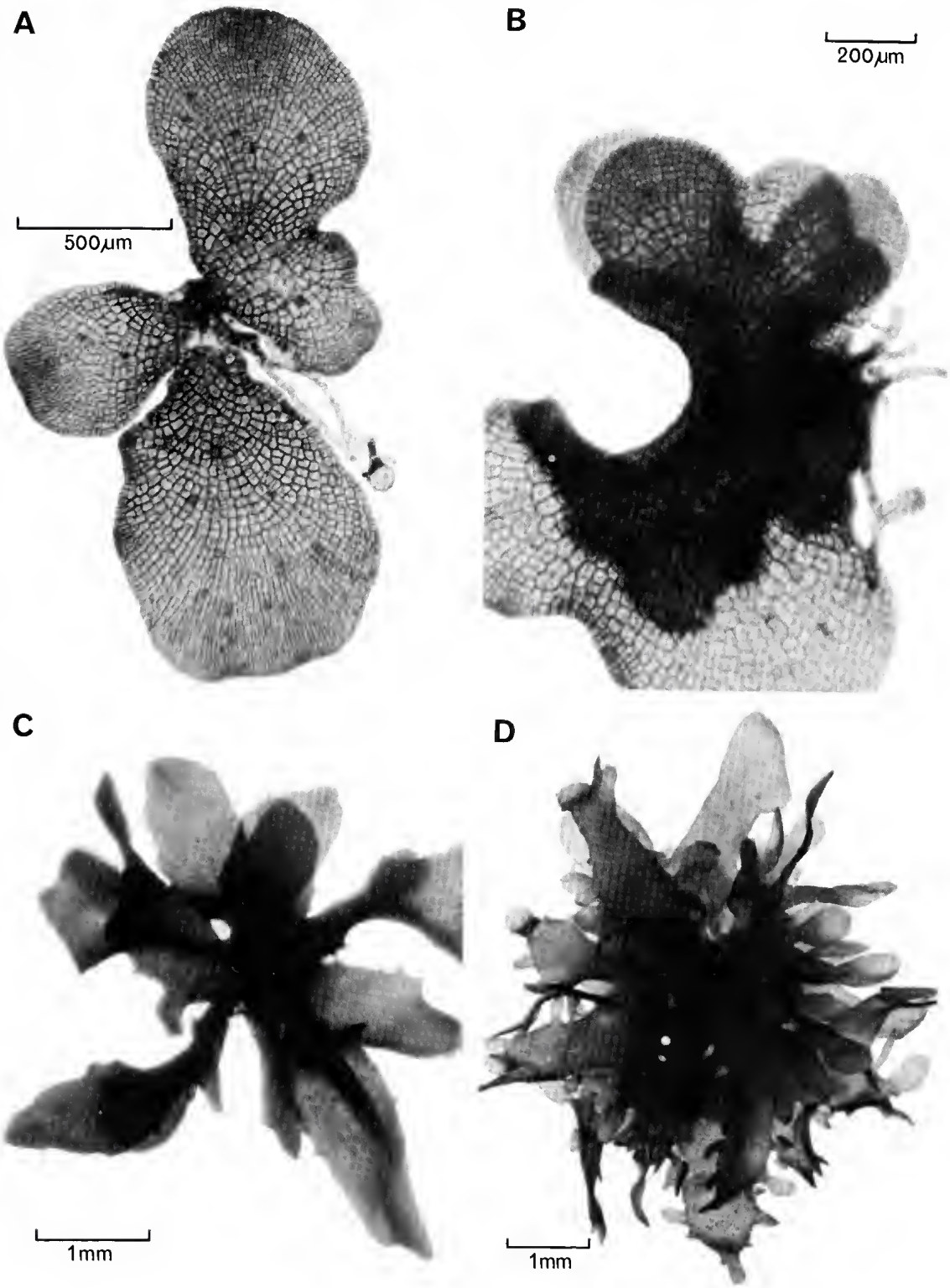

Fig. 4.

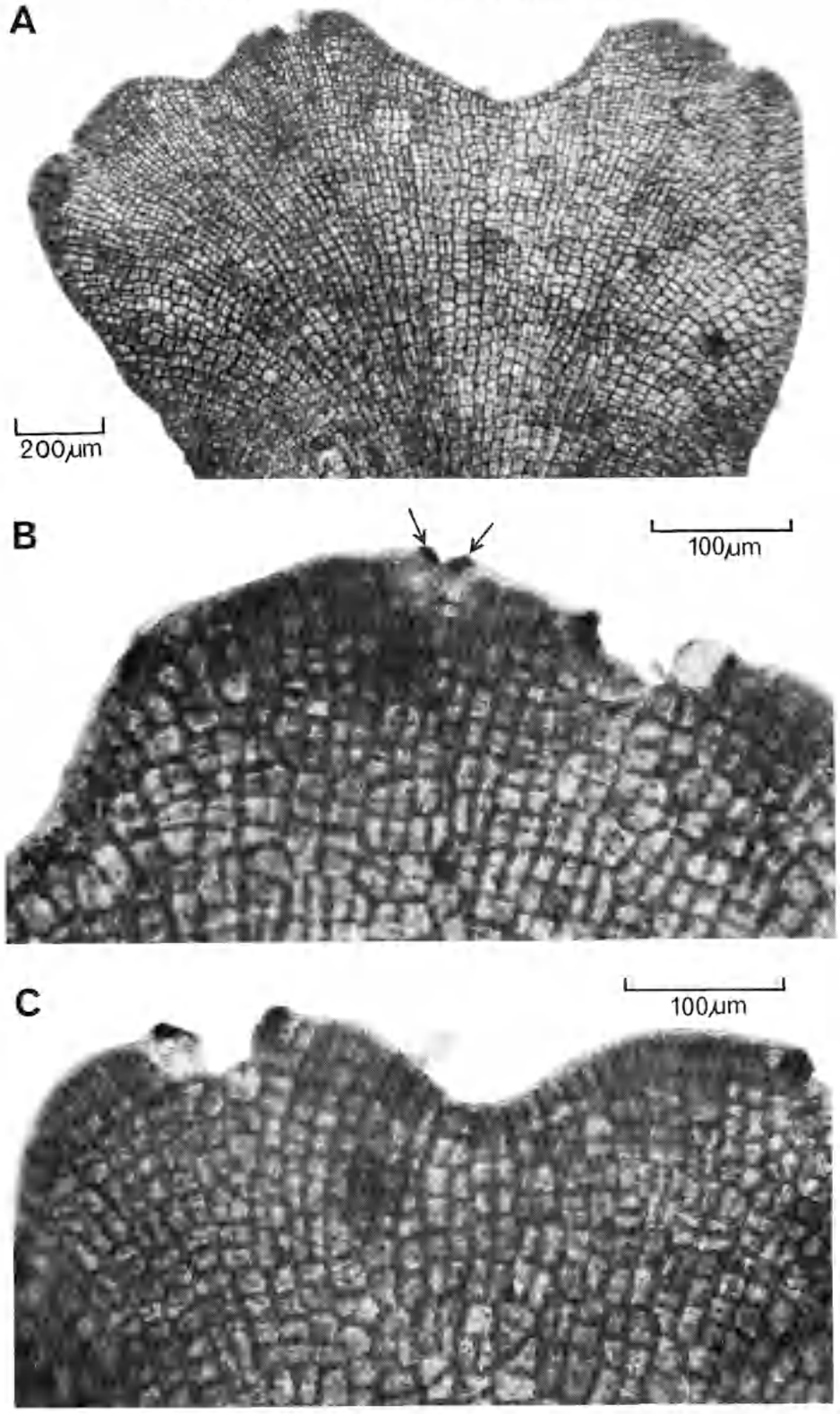

Fig. 5.

Fig. 2. *A*. Thallus of *Lobospira*, showing basal entangled branches with tendril-bearing laterals, and erect branches with ultimate bicuspid ramuli. Stanley Beach, Kangaroo I., S. Aust. (*Edelstein & Kraft* 126, 25.iv.1972).
B. Surface view of a branch showing tetrasporangia (undivided) and released tetraspores (arrow). (ADU, A42264.)

Fig. 3. *A*. A multicellular sporeling, with rhizoid, about 2 weeks old.
B. A plant about 3 weeks old, showing development of a flat thallus with a meristematic margin.
C. Plant 6 weeks old, with two lobes.
D. Plant 6 weeks old, showing development of lobes from the margin and especially from the rhizoidal region.

Fig. 4. *A*. Plant 7½ weeks old, with 3 main lobes.
B. Plant 7½ weeks old, becoming convolute.
C. Plant 12½ weeks old, with several irregular lobes.
D. Plant 17½ weeks old, consisting of many irregular branches, each with marginal lobes of various sizes.

Fig. 5. *A*. Plant 24½ weeks old: general view of meristematic apex, with degenerating cells in centre of each lobe.
B. As in *A*, with two darkly-staining cells (arrows) separating in centre of margin of lobe.
C. As in *A*, with degeneration of cells between two darkly-staining cells.

dium was changed every 3-5 days for the first 6 weeks and thereafter at weekly intervals.

Results

Development of the thallus

Dissection of apices (Fig. 1*A*) of mature field plants of *Lobospira* shows that development is from a row of apical cells, from which lateral groups of apical cells separate off alternately and differentiate rapidly to form a larger, abaxial, spinous or bicuspid process with a group of meristematic cells on its adaxial side. Growth is thus monopodial and the affinities of *Lobospira* are with the Zonarieae. In actively growing apices, the young laterals develop rapidly and overtop the apex. The meristematic group of cells on the lateral may persist indefinitely, in which case a long lateral develops; or it may persist to give a short lateral with only a few pointed ramuli; or it may not develop further, resulting in only a pointed or bicuspid ramulus, at the apices of which a single cell remains prominent (Fig. 1*A*). Alternate series of these ramuli fringe the longer axes or branches.

The indefinite axes twist spirally so that the branches or ramuli become arranged with a phyllotaxis of about 1/3.

The mature thallus consists of a cortical layer of cells which are arranged more or less in longitudinal lines and tend to radiate upwards. The medulla consists of cells of similar size but rather irregularly arranged (Fig. 1*B*), and is 2–5 cells thick; in older branches a slight midrib is present where the medulla is thicker. Older axes are ovoid to round in section and a central core of narrower and more elongate cells may be present. Hair groups are of frequent occurrence on the thallus.

Sporangia and spore development

Division of the sporangia into four, tetrahedrally arranged, non-motile spores (Fig. 2*B*) appears to occur only shortly before their release, and no divided sporangia have been observed in any herbarium material. Fertile collections have been made mainly in autumn (April to June).

The spores germinated on slides within two days of their release, forming a short rhizoid which was cut off by a cross wall when slightly longer than the spore. The rhizoid became 3 or 4 cells long before the spore-residual cell enlarged and divided. By day 7, a variety of cell arrangements (Fig. 1*C*) was present amongst the sporelings, which, during the next 2 weeks, developed into elongate-ovoid masses of cells (Figs 1*D*, 3*A*), their arrangement depending on the early cell divisions.

From this cell mass, which was attached by a relatively long rhizoid of several cells, a flat, ovate-spathulate disc of cells developed, mostly two cells thick and with a distinct apical row of meristematic cells (Fig. 3*B*, plant 3 weeks old). During the next 4–5 weeks, further rhizoids developed from the basal cell mass, and the flat, erect, frond developed further from the apical meristem, usually becoming lobed (Fig. 3*C*). Smaller lobes developed both from the

basal cell mass and lower parts of the erect fronds (Fig. 3*D*).

By 8 weeks, the plants had developed several fronds (Fig. 4*A*) of varying sizes and often the main frond was becoming convoluted (Fig. 4*B*). Tufts of long, colourless hairs differentiated at this stage of development. By 12 weeks, numerous fronds were present (Fig. 4*C*), usually branched or lobed, and thalli 17½–24½ weeks old formed a cluster of fronds (Fig. 4*D*) up to 1 cm across. One plant reached almost 2 cm across after 34 weeks but showed no further morphological development. At this stage, all thalli were heavily overgrown with epiphytes and died. Apart from the meristematic and lateral margins, the fronds were mostly two cells thick, increasing to 3 or 4 cells thick in the older parts.

The apical marginal row of meristematic cells was prominent in all branches and lobes, giving a typical "zonarioid" appearance (Fig. 5*A*). Plants 24½ weeks old showed a further apical development of possible significance, in that centrally along the meristematic margin of each lobe, two cells became more prominent (Fig. 5*B*) with denser protoplasm, and breakdown of tissue occurred between them (Fig. 5*A*, *C*). Whether this was only a breakdown feature before death is uncertain, but the two cells concerned, which when first noticeable were densely protoplasmic and appeared healthy, could possibly correspond to the single cells which are prominent at the apex(ices) of the single or bicuspid ramuli of the mature plant.

Conclusions

The division of sporangia to give four nonmotile spores, and the occurrence of a marginal row of apical cells in both the adult axes and in juvenile stages, indicate that *Lobospira* is correctly placed in the Dictyotales, but belongs in the Zonarieae and not the Dictyoteae. The distinctive morphology of *Lobospira* separates it generically from all other genera of the Zonarieae.

While the sporeling and juvenile stages are now known, further studies are necessary to show how such stages develop to the mature laterals which cut off pointed or bicuspid ramuli. Since sexual plants are still unknown, cytologcial studies on the division of the sporangia are desirable to indicate whether meiosis occurs at this stage.

Acknowledgments

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